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Less Than 40% of the Simian Virus 40 Large T-Antigen-Coding Sequence Is Required for Transformation

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F8*dl* is a simian virus 40 early-region deletion mutant that lacks the simian virus 40 DNA sequences between 0.168 and 0.424 map units. Despite this large deletion, cloned F8*dl* DNA transforms Fisher rat F111 cells and BALB/3T3 clone A31 mouse cells as efficiently as does cloned simian virus 40 wild-type DNA. These results indicate that less than 40% of the large T-antigen-coding sequence is required for efficient transformation.

For some time, it has been assumed that an intact simian virus 40 (SV40) early region is required for the transformation of cells in culture (10). Recently, however, two groups have reported that SV40 mutants with substantially deleted early regions are able to transform, although with greatly reduced efficiencies (about 0.1 to 1.0% that of wild-type SV40) (1, 6, 8). We report here that the mutant F8*dl* transforms Fisher rat F111 and BALB/3T3 mouse cells as efficiently as does wild-type SV40, even though it lacks more than 60% of the DNA sequences normally used to encode large T antigen. Our results suggest that the domain of large T antigen essential for transformation is encoded by the sequences between 0.424 and 0.644 map units.

In an attempt to understand the mechanism by which SV40 transforms cells in culture, we have constructed an SV40 deletion mutant that lacks the early-region sequences between 0.168 and 0.424 map units (7). This mutant, F8*dl*, encodes a normal small t antigen and several truncated forms of the SV40 large T protein, the largest of which has a molecular weight of about 34,000 (34K). We cloned the DNA of F8*dl* into pBR322 at the *Bam*HI site and used this cloned DNA to infect Fisher rat F111 cells. We suspended the infected cells in soft agar and 8 days later counted the number of small, abortively transformed colonies. Four weeks after suspending the cells in agar, we scored the number of large, stably transformed colonies. We used this agar assay because anchorage-independent growth is generally regarded as the most stringent test for transformation. The results of this experiment are shown in Table 1. Here we see that cloned F8*dl* DNA (pF8*dl*) is as efficient as cloned wild-type DNA (pWT) in inducing both abortive and stable transformation of F111 cells.

We have previously shown that F8*dl* encodes truncated forms of large T antigen with molecular weights of about 34K, 24K, 22K, and 20K (7). For example, Fig. 1 is a fluorogram of labeled proteins extracted from BSC-1 cells infected with F8*dl* plus a *d*1884/*ts*B4 double-mutant helper. This figure shows that when proteins from these productively infected cells are precipitated with anti-SV40 tumor serum, the F8*dl*-encoded, truncated forms of T antigen are readily observed. To test whether or not the F8*dl* transformants expressed these characteristic proteins, we removed five large colonies from F8*dl*-transformed agar cultures and expanded them into cell lines. We then used anti-SV40 tumor serum to precipitate labeled proteins extracted from the F8*dl*-transformed lines. Figure 2 shows that the expected truncated forms of large T antigen are present in all five mutant transformants, suggesting that one or more of these truncated antigens is required for transformation. We then tested these mutant-transformed lines to determine whether they retained the ability to grow in soft agar. Table 2 shows that all five lines grow in agar, demonstrating that the transformed phenotype is stably expressed.

Next we assayed the ability of cloned F8*dl* DNA to transform BALB/3T3 clone A31 cells to anchorage-independent growth. F8*dl* transforms these mouse cells both abortively and stably with wild-type efficiency (Table 3). Thus, the ability of F8*dl* to transform is not limited to rat cells.

In an earlier publication, we reported that F8*dl* DNA was able to transform C3H10T1/2 mouse cells but with a frequency that was only about 1% that of wild-type SV40 DNA (5, 8). This result, which contrasts sharply with the studies presented here, might be explained if F8*dl* DNA were inefficiently integrated into the genomes of C3H10T1/2 cells. To test this idea, we infected C3H10T1/2 cells with cloned F8*dl* DNA, suspended these cells in soft agar, and 8 days later counted the number of small, abortively transformed colonies. Since this assay measures the transient expression of the transformed phenotype, a stable association between viral and cellular genomes is not required (9). Thus, even if F8*dl* were defective in a viral integration function, we would still expect the mutant to induce abortive transformation with wild-type efficiency. The results of this experiment (Table 4) show that the efficiency with which F8*dl* DNA

TABLE 1. Abortive and stable transformation of F111 cells

DNA used for infection[a]	% Abortive transformation[b]	% Stable transformation[c]
Salmon sperm	0.02	<0.002
pF8*dl*	0.50	0.09
pWT	0.40	0.14

[a] Petri dishes (35 mm) were seeded with 3×10^5 cells, and about 6 h later, these cells were infected with 6 μg of the appropriate DNA per dish by the modified calcium phosphate technique (3, 11).

[b] About 24 h after infection, the infected cells were suspended in 0.34% agar–Dulbecco modified Eagle medium–20% calf serum. The cultures were incubated at 40.5°C for 8 days, and the number of colonies that contained more than three cells was counted. About 50,000 cells were scored for each entry. We used 40.5°C for these assays because F111 cells grow faster at this temperature than they do at 37°C. Similar results were obtained at 37°C, but the stable transformation assays require almost a week longer to develop.

[c] Approximately 4 weeks after the cells were suspended in agar, the number of colonies that had greater than 20 cells was counted. No such colonies arose on mock-infected cultures (salmon sperm DNA).

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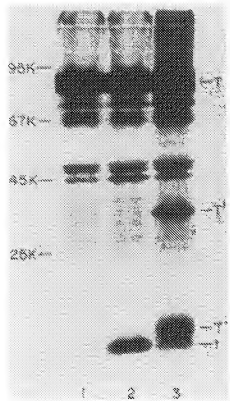

FIG. 1. Tumor antigens expressed in F8*dl*-infected BSC-1 monkey cells. BSC-1 cells were infected with *dl*884/*ts*B4 helper virus (lane 1), wild-type SV40 (lane 2), or a mixed lysate of F8*dl* virus plus the *dl*884/*ts*B4 helper (lane 3). Two days after infection, the cells were labeled for 2 h with [^{35}S]methionine (0.4 mCi/ml, 1,000 Ci/mmol). We extracted labeled proteins, immunoprecipitated with anti-SV40 tumor serum, and analyzed the precipitates by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (6 to 15% gradient) (2, 4). The positions of the F8*dl*-encoded truncated tumor antigens are indicated by T'.

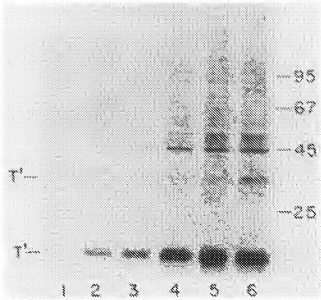

FIG. 2. Tumor antigens expressed in F8*dl*-transformed F111 cells. We labeled F111 cells (lane 1) and F111 cells transformed by F8*dl* (lines 401 to 405 in lanes 2 to 6, respectively) with [^{35}S]methionine (0.4 mCi/ml, 1,000 Ci/mmol) for 2 h, extracted proteins, immunoprecipitated these labeled proteins with anti-SV40 tumor serum (2), and subjected these immunoprecipitates to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (4). A fluorogram of this gel is shown here. The positions of the F8*dl*-encoded truncated tumor antigens are indicated by T'.

abortively transforms C3H10T1/2 cells is reduced at least 60-fold relative to that of wild-type SV40 DNA. Therefore, the low frequency with which F8*dl* transforms C3H10T1/2 cells is not due simply to inefficient viral integration.

A more likely explanation for the low efficiency with which F8*dl* transforms C3H10T1/2 cells is that in this line, only a small fraction of the cells express the F8*dl* transforming protein(s) at levels great enough to effect transformation. Although we have no direct evidence to support this hypothesis, several results suggest that this explanation may be correct. In preliminary experiments, we have found that the F8*dl*-encoded, 34K truncated tumor antigen is located in the nucleus and in the plasma membrane, whereas the 20K, 22K, and 24K truncated antigens are primarily cytoplasmic (L. Sompayrac, unpublished data). Since the localization of the 34K protein resembles that of wild-type T antigen, this 34K protein is most likely to be the F8*dl* transforming protein. However, in F8*dl*-transformed lines, we routinely observe that the levels of the 34K protein are low when compared with the levels of the smaller truncated forms of T antigen (for example, see Fig. 2 or reference 8). This is because each of the F8*dl* truncated proteins is encoded by a uniquely spliced RNA, and the message for the 34K protein is underrepresented relative to the messages for the 20K to 24K proteins (L. Sompayrac, unpublished data). As a result of the way in which the RNA is partitioned, the level of 34K protein expression may be too low to efficiently transform some cell lines. Further, all of the F8*dl*-transformed C3H10T1/2 mouse lines we have examined have multiple inserts of viral DNA, whereas the wild-type-transformed C3H10T1/2 lines have single inserts (8). This result suggests that a high gene dose of F8*dl* DNA may be required for transformation in this cell line.

In summary, F8*dl* is able to transform two widely used cell lines with wild-type efficiency, even though it lacks more than 60% of the DNA sequences that normally encode large T antigen. This result implies that in these lines the SV40 sequences essential for transformation are located between

TABLE 2. Growth in agar of F8*dl*-transformed F111 cells

Cell line	Transformed by:	% Cells that grow in agar[a]
F111	Untransformed	0
407	pWT	9
408	pWT	42
411	pWT	25
401	pF8*dl*	12
402	pF8*dl*	10
403	pF8*dl*	62
404	pF8*dl*	43
405	pF8*dl*	27

[a] Cells from each line (3×10^4 per 60-mm dish) were suspended in the same agar medium as that used for transformation assays (Table 1). About 3 weeks later, the percentage of cells that had grown into colonies with greater than 20 cells was counted.

TABLE 3. Abortive and stable transformation of BALB/3T3 cells

DNA used for infection	% Abortive transformation[a]	% Stable transformation[b]
Salmon sperm	0.013	<0.003
pF8*dl*	0.13	0.04
pWT	0.17	0.03

[a] Conditions were the same as those in Table 1, except the cultures were incubated at 39°C and the fraction of the colonies with greater than four cells was scored.

[b] No stably transformed colonies arose on mock-infected cultures (salmon sperm DNA).

TABLE 4. Abortive transformation of C3H10T1/2 cells

DNA used for infection	% Abortive transformation[a]
Salmon sperm	0.01
pF8*dl*	0.008
pWT	0.69

[a] Conditions were the same as those in Table 1, except the cultures were incubated at 37°C.

0.424 and 0.644 map units, since these are the only early-region sequences present in F8*dl*.

Because of its large early-region deletion, F8*dl* can be expected to have lost many functions required for the SV40 lytic cycle. We have shown, for example, that F8*dl* is defective for viral DNA replication (7). Thus, since F8*dl* can transform, this mutant should be a useful tool to distinguish between functions essential for transformation and functions required only for the lytic cycle.

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